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Hypothermia has been and continues to be a problem faced by military personnel when operating in cold environments or when water immersion in moderate temperatures results in the loss of body heat that is not compensated for by heat production. Vascular leakage of large protein molecules and accompanying water or extravasation manifested in a loss of vascular volume is one of the pathophysiological findings resulting from rewarming following hypothermia. Recent work in this laboratory with hyperthermic rats has shown that hyperthermia-induced extravasation can be quantitated and the organ sites located using recovery of Evans Blue from tissues. Recovery of fluorescent microspheres from the same rats indicated a change in reticuloendothelial system function with hyperthermia. Prior to using the same techniques to quantitate these changes following hypothermia and rewarming, it was necessary to develop a non-invasive method of inducing closely controlled hypothermia in rats. This report describes the construction of three water cooled copper coils into which anesthetized rats were placed. Valves in the parallel tubing connecting these coils allowed the cooling and subsequent rewarming of three rats simultaneously with only one circulating water bath. Data on 3 sets of 3 rats are presented.

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Non-invasive method of inducing hypothermia in multiple rats simultaneously

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CONTENTS

LIST OF FIGURES

EXECUTIVE SUMMARY

Hypothermia has been and continues to be a problem faced by military personnel when operating in cold environments or when water immersion in moderate temperatures results in the loss of body heat that is not compensated for by heat production. Vascular leakage of large protein molecules and accompanying water or extravasation manifested in a loss of vascular volume is one of the pathophysiological findings resulting from rewarming following hypothermia. Recent work in this laboratory with hyperthermic rats has shown that hyperthermia-induced extravasation can be quantitated and the organ sites located using recovery of Evans Blue from tissues. Recovery of fluorescent microspheres from the same rats indicated a change in reticuloendothelial system function with hyperthermia. Prior to using the same techniques to quantitate these changes following hypothermia and rewarming, it was necessary to develop a non-invasive method of inducing closely controlled hypothermia in rats. This report describes the construction of three water cooled copper coils into which anesthetized rats were placed. Valves in the parallel tubing connecting these coils allowed the cooling and subsequent rewarming of three rats simultaneously with only one circulating water bath. Data on 3 sets of 3 rats are presented.

INTRODUCTION

Hypothermia- Historically, cold ambient temperatures have resulted in substantial numbers of military casualties due to exposure to the elements resulting in hypothermia. Recent events have served to demonstrate that the problems of cold exposure still hamper soldier effectiveness. During February 1995, four Ranger students died of hypothermia while undergoing partial immersion in swamp training at Camp Rudder, Pensacola, Florida. British troops sustained numerous hypothermia-related injuries in the Falklands in 1992, and the current operating conditions of our soldiers in Bosnia expose them to the risks of hypothermia.

Accidental hypothermia is an acute drop in core temperature (Tcore) usually in cold environments without any pathology of the thermoregulatory system. Hypothermia is more common in the elderly (reduced metabolic rate and insensitivity to cold) or the very young (increased heat loss do to a large surface area to mass ratio), but it is also seen in fit individuals working to exhaustion in cold environments (2,18,22). As body temperature falls there is a decrease in blood flow to the extremities to conserve heat in the heart and brain. Also, shivering is initiated in an attempt to increase metabolic heat production. In humans, mild hypothermia (Tcore 34-36°C) is characterized by increases in heart rate, peripheral vascular resistance, blood pressure (BP), and cardiac output (CO). Moderate hypothermia (Tcore 30-34°C) is characterized by decreases in heart rate, BP, and CO as well as the occasional appearance of arrhythmias. Severe hypothermia (Tcore <30°C) is characterized by intensifying symptomatology and the appearance of the characteristic "J" or Osborn wave in the ECG (22). Patients with severe hypothermia may appear clinically dead with marked decrease in cerebral blood flow, CO and arterial pressure.

Clinical Uses of Hypothermia- While controlled mild to moderate hypothermia is commonly used during many surgical procedures to lower metabolic rate and reduce the dangers of ischemia during temporary diversion of the blood supply from target organs (1,8), deep hypothermia can have serious pathophysiological consequences. In a clinical setting moderate hypothermia has reduced the severity of damage from traumatic brain injury in humans (12) and mild hypothermia decreased spinal cord injury in rabbits (13).

Hypothermic Pathophysiology- Rewarming from accidental severe hypothermia is associated with sudden, often fatal, vascular collapse characterized by falling CO and sudden drops in peripheral resistance and BP (16,20,21). During severe hypothermia in rats there is a decrease in blood flow to brain, heart, liver, spleen, kidney, intestines, and muscle to 3-33% of normal values; even upon rewarming these flows may still be depressed to 5-55% of pre-hypothermic values (21). Cooled blood circulating through the brain disrupted the blood brain barrier (15) while hypothermia altered endothelial cell structure (8). Cold exposure results in loss of transmembrane ion gradients and barrier function. Ion pump failure results in increased Ca^{++} concentration and consequent cytoskeletal disruption followed by organ damage due to the breakdown of endothelial integrity (8). Exposure to cold air results in epithelial damage and vascular leakage in the respiratory system (7,24). An increase in the viscosity of the blood results in extravasation of plasma proteins and water (25). This loss of fluid from the vascular bed is postulated to be responsible for the diminished CO (1) which may result in terminal

vascular collapse. Work on an isolated cat limb model (23,25) indicates that there is extravasation in peripheral vasculature, but no attempt was made to evaluate extravasation in specific organs or to ameliorate the problem.

Animal models of hypothermia- Many methods have been described for cooling the rat (17): hypercapnic hypoxia followed by placement in a glass chamber in a cold water bath; maintenance in a 4°C environment following injection of chlorpromazine sc 20 mg/kg; immersion to the neck in an ice water bath; cooling after insulin injection by confinement in a water bath or cold room; starvation for 2 or more days before cooling; cooling with sodium pentobarbital (17). In addition to chlorpromazine (5,9,11,17) L-tryptophan (3,5), salicylate (4), tyrosine (4), and 5-thio-D-glucose (6) have all been used to induce hypothermia by drug administration combined with cold exposure.

In a study of auditory evoked responses, rats were made hypothermic (31.5°C) by administration of sodium pentobarbital followed by exposure to Tamb= 4°C until the desired Tcore was achieved (10). Halothane anesthesia and a cold water blanket were used to induce hypothermia to examine effects on amino acid release following ischemia (14). In a more extreme study, rats were anesthetized with sodium pentobarbital and Tcore reduced to 13°C with ventilatory support below 20°C by insertion of U-shaped PE tubing into the rectum and esophagus through which water circulated for cooling and rewarming (20,21).

For this study, it was necessary to develop a method of non-invasively inducing hypothermia in rats with minimal stress. Invasive methods, immersion studies, or drug- induced methods have potential for altering vascular permeability, reticuloendothelial system function, or the blood pressure, heart rate and ECG measurements planned for future studies.

MATERIALS AND METHODS

Animals- Adult male (N = 9, 490 ± 27g) Sprague-Dawley rats, (Harlan Sprague Dawley, Inc. Indianapolis, IN) were housed in the USARIEM animal colony until the start of the experiment. The rats were maintained at 26°C and 50% rh and were caged individually in wire bottomed cages. Lighting was controlled automatically (on, 0600-1800 h) and Purina rat chow and water were available ad lib except during the experimental interval. The ambient temperature in the experimental room was 20-23°C.

Cooling apparatus- The individual coils (Fig. 1) into which the rats were placed were constructed of ¼" O.D. copper refrigeration tubing. The tubing was bent by hand into a coil of 14 turns; the opening at the large end through which the rat enters is 3.5" and at the small end is 2", requiring approximately 7 ft of tubing for each coil. The coil thus formed may be easily expanded or contracted to accommodate different sized animals.

The coil is supported on a platform (Fig. 1) made of plexiglass and stainless steel rods; this is derived by removing rods from a previously described (19) restraining cage. The 3 coils are connected to a water bath in parallel (Fig. 2) using vinyl tubing (¼" I.D.), "Y" and "T"

connectors, and hose clamps. A stopcock was placed at the inlet of each coil to regulate flow to each coil individually. Surface thermistors were placed at the inlet of each coil.

Cooling procedure- Rats were lightly anesthetized (sodium pentobarbital, 35 mg/kg, ip); 15 min after anesthesia administration, a rectal probe (Tcore) was inserted 6 cm beyond the anal sphincter and taped in place, and then each animal was placed in a coil. The coil containing the rat was then lightly wrapped with "bubble wrap" to provide insulation that neither prevented observation of the animal nor interfered with respiration.

Hypothermia was induced by a ramped drop in the temperature of the water from the water bath. The bath temperature (Tbath) was started at 15°C and then the temperature setting dropped to 10, 5 and finally 3°C at 5 to 10 min intervals. The water bath lagged behind the setting so that the water entering the coil did not reach 5°C until 30 min into the cooling procedure. The coil temperature (Tcoil) was never lower than 5°C indicating that there was a 2°C rise in water temperature between the water bath and the coil. Rats were taken to a Tcore as low as 19°C and maintained there for 30 min when a staged increase in the Tbath was used to rewarm the animals.

RESULTS

Figures 3-5 each represent the cooling curves of 3 rats cooled simultaneously on 3 different days. At time 0 (Fig. 3), Tbath was 15°C; over the next 15 min. the Tbath set point was moved to 5°C, but the water entering the coils (Tcoil) did not stabilized at 7°C until 30 min. At 45 min the Tbath set point was lowered to 3°C and Tcoil stabilized at 5°C by 60 min. At 80 min. the Tcore of rat 98218 was dropping at a faster rate than that of the other 2 animals; therefore, the flow into its coil was stopped which decreased the rate of drop of Tcore. At 100 and 110 min. the flow was terminated for #'s 98217 and 98219 respectively. The lowest Tcore attained was 23.4°C for 98218 at 100 min. At 120 min. the Tbath was increased to 25°C and at 125 min the flow was resumed in all coils. At 130 min Tbath was increased to 30°C and at 150 min was changed to 35°C. By 180 min. all animals had regained a Tcore of at least 35°C and were fully recovered from anesthesia.

In Fig. 4, the Tcore of 3 additional animals and the Tcoil are illustrated. The Tbath was decreased from 15°C to 3°C over the first 20 min. with the water entering the coil stabilizing at 5°C at 30 min. Rat 98234 cooled faster than the others; at 40 min. the flow to its coil was stopped, the insulation removed, and Tcore was maintained at the same level for the next 30 min. When 98235's Tcore approached that of 98234, flow was resumed in 98234's coil and its Tcore then tracked that of the other animals. At 120 min., flow was stopped in all coils to maintain the Tcore's around 23°C for 30 min after which the Tbath was raised to 25°C, the flow restarted, and then Tbath ramped up to 35°C.

On the third trial day (Fig. 5), Tcore's of all animals were closely modulated by earlier control of flow. When Tcore of 98232 began to fall faster at 120 min, it was allowed to fall to 20°C, the lowest Tcore to be used in future work. Rewarming was as in Fig. 3 and 4.

DISCUSSION

Results presented demonstrate the uniform induction of hypothermia in 3 rats simultaneously. Figures 3-5 clearly indicate that it is possible to control finely each animal's Tcore drop to align rats by modulating flow through the coil and removal of the insulation. Thus, with a single water bath, 3 rats were cooled simultaneously and consistently. Carefully controlled hypothermia will be important to upcoming studies on the pathophysiological effects of deep hypothermia and rewarming.

In another pilot experiment, an animal equipped with a surgically implanted telemetry transmitter (Data Sciences) was anesthetized and placed in one of the coils. During the cooling of this animal (data not shown), the copper coil did not interfere with the telemetry signals. Therefore, this system will be used to obtain blood pressure, heart rate, and ECG in addition to body temperature during induction of hypothermia.

This method for inducing closely controlled rate, duration, and intensity of hypothermia will be used to quantitate hypothermic effects on extravasation and reticuloendothelial system function. Subsequently, this system will be ideal to evaluate potential pretreatment and treatment modalities for stabilizing the vasculature to prevent extravasation of critical volume and to aid in preventing the vascular collapse and potential mortality that may result on rewarming from hypothermia.

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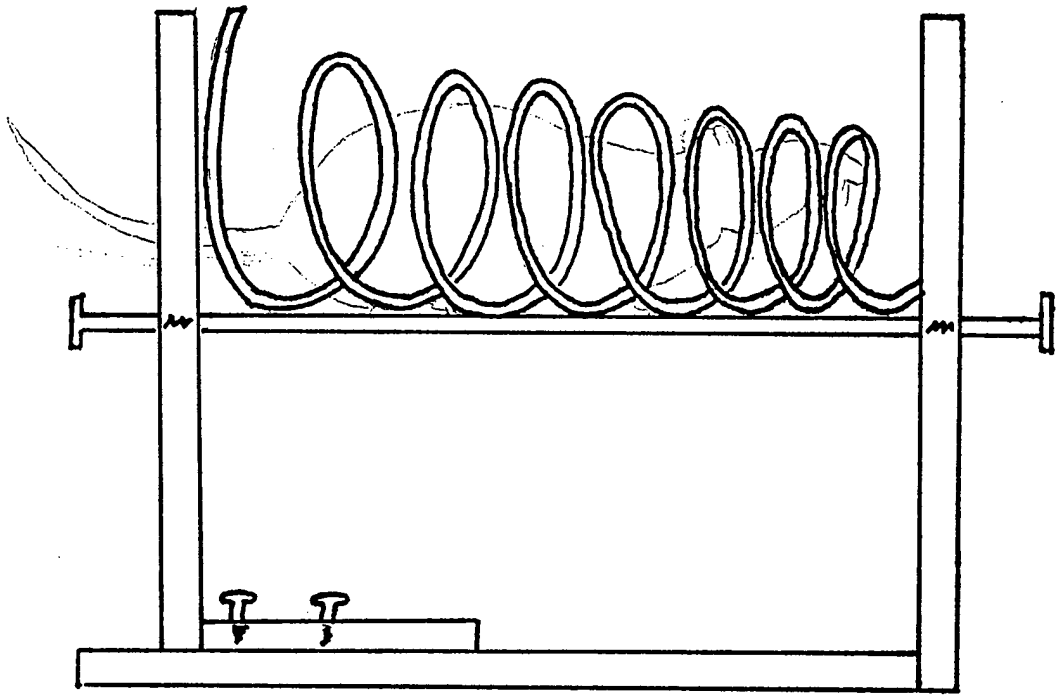
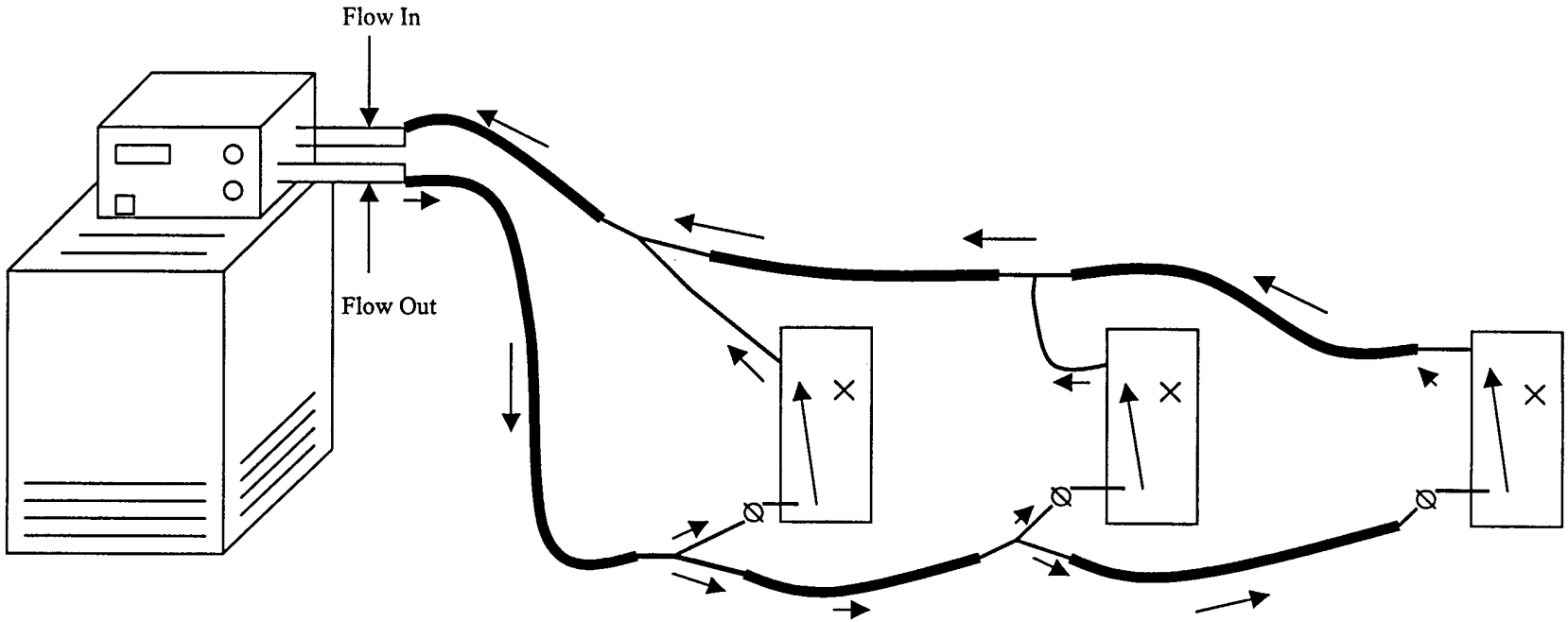


Fig. 1- The individual coils into which the rats were place were constructed of ¼" O.D. copper refrigeration tubing. The tubing was bent into a coil of 14 turns. The coil is supported on a platform made of plexiglass and stainless steel rods derived by removing rods from a previously described (16) restraining cage.



X- Coiled copper tubing through which water circulates.
⊘ - Stopcock for adjusting waterflow.

Fig. 2- This is a schematic of the 3 rat cooling system. The 3 coils are connected to a water bath in parallel using vinyl tubing, "Y" connectors, and hose clamps. A stopcock was placed at the inlet of each coil to regulate flow to each coil.

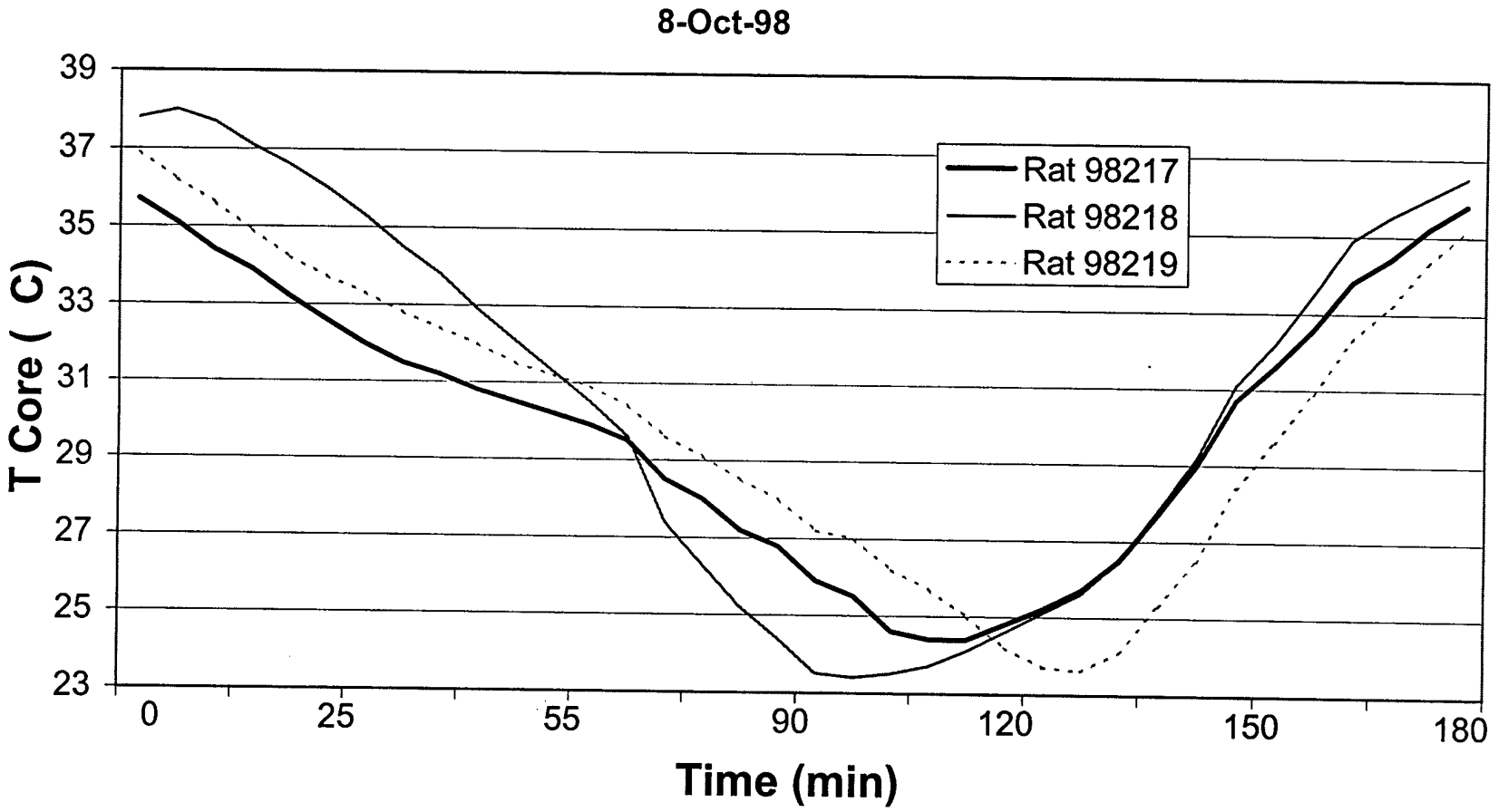


Fig. 3- Figures 3 represents the cooling curves of the 3 rats cooled simultaneously 8 Oct 1998. At time 0, Tbath was 15°C; over the next 15 min. the Tbath setpoint was moved to 5°C. At 45 min the Tbath setpoint was lowered to 3°C and Tcoil stabilized at 5°C by 60 min. The lowest Tcore attained was 23.4°C for 98218 at 100 min. At 120 min. the Tbath was increased to 25°C, at 130 min to 30°C, and at 150 min to 35°C.

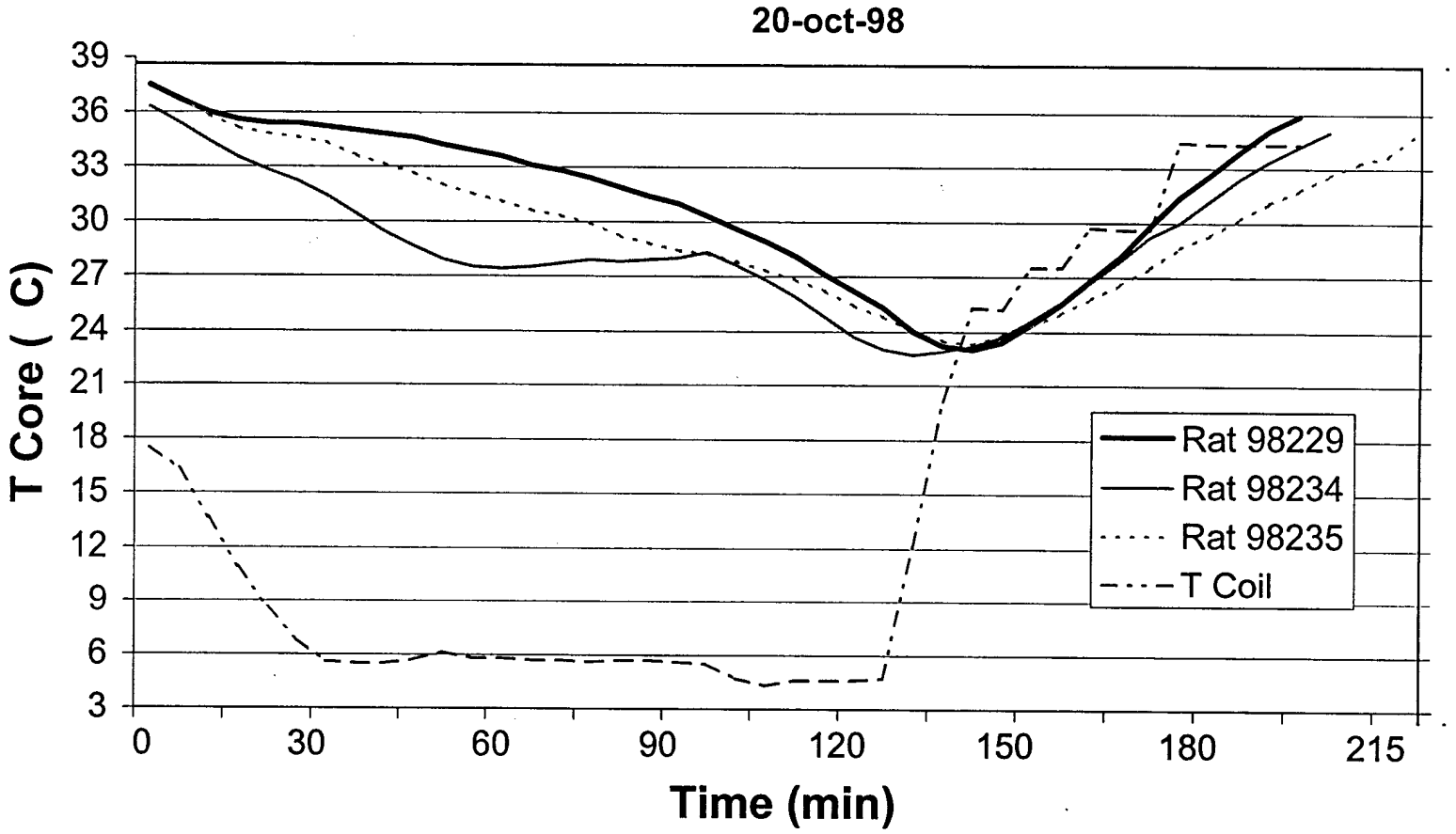


Fig. 4- Figure 4 represents the Tcore of 3 animals as well as the Tcoil for the 20 Oct 98 trial. The Tbath was decreased from 15°C to 3°C over the first 20 min. with the water entering the coil stabilizing at 5°C at 30 min. At 120 min., flow was stopped in all coils to maintain the Tcore's around 23°C for 30 min after which the Tbath was raised to 25°C, the flow restarted, and then Tbath stepped up to 35°C.

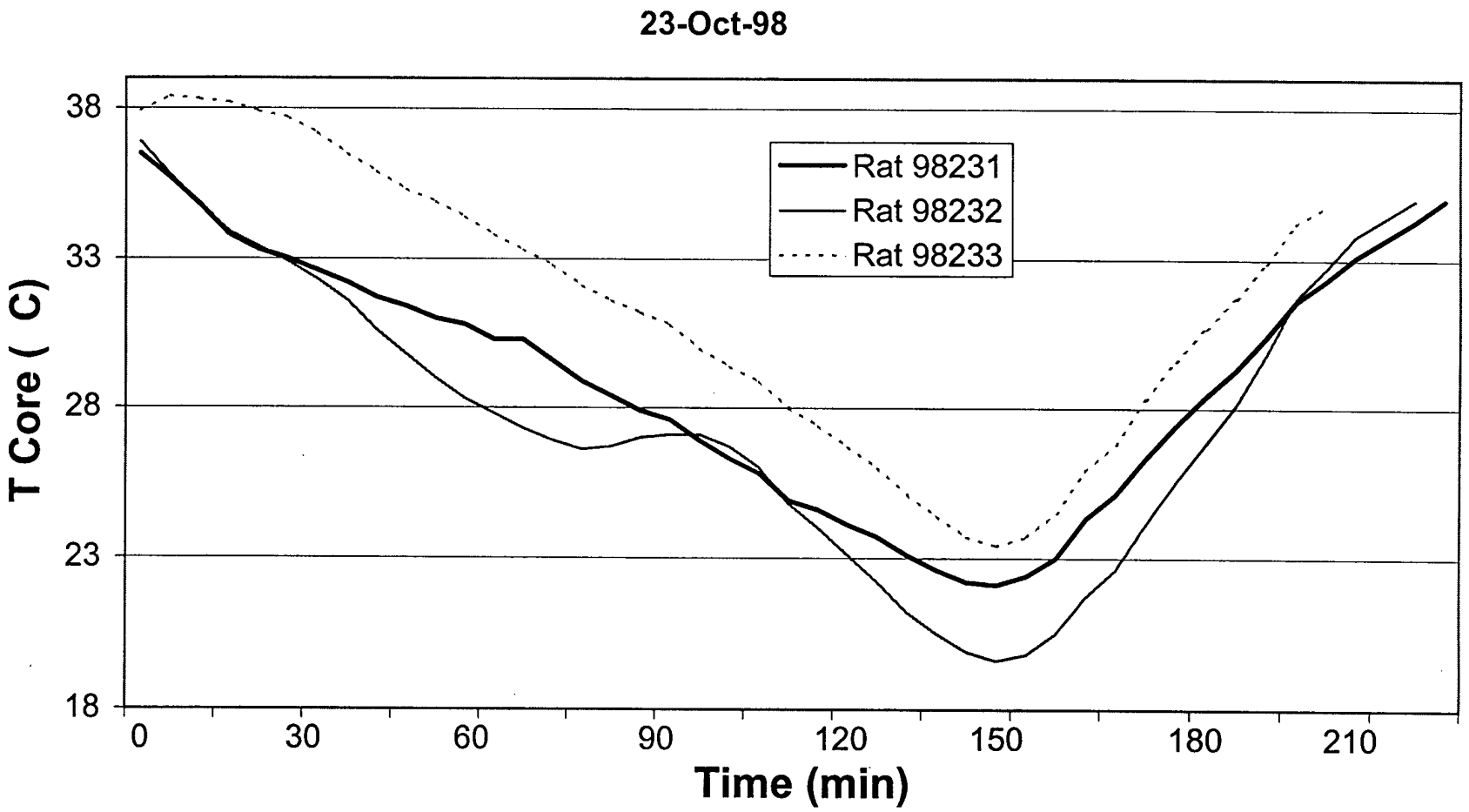


Fig. 5- Figure 5 represents the Tcore of the 3 animals cooled on 23 Oct 1998. Tbath was again decreased from 15°C to 3°C over the first 20 min. All animals were kept closer together than in previous trials by earlier modulation of flow into the coils. When Tcore of 98232 began to fall faster at 120 min, he was allowed to fall to 20°C. Rewarming was as in Fig. 3 and 4.

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